

Redoing AI0

	PCR 20 µL reac	Final Concentration			
10x thermopol reaction b	2.5	1X			
10 mM dNTPs	0.5	200 µM			
10 µM Forward Primer	0.5	0.2 µM			
10 µM Reverse Primer	0.5	0.2 µM	Primers:	r_Squ_ADH1gRNA	
Template (colony)	0	5 ng		f_Squ_ADH1gRNA	
DMSO (optional)	0	3 %			
Taq polymerase	0.125	1.0 units/50 µl PCR			
Nuclease-free Water	20.875				
Total volume	25				
Number of Reactions	17	15x1 samples + 1 negative control +1 extra			AI0-11
					AI0-12
10X Reaction buffer	42.5				AI0-13
dNTPs	8.5				AI0-14
r_Squ_ADH1gRNA	8.5				AI0-15
f_Squ_ADH1gRNA	8.5				AI0-16
Taq Polymerase	2.125				AI0-17
Water	354.875				AI0-18
Total volume	425				AI0-19
Volume per tube	25				AI0-20
					AI0-21
					AI0-22
PCR program A 'Y-COL-					AI0-23
Initial Denaturation	95°	7 min			AI0-24
	95°	30 sec			AI0-25
	52°	1 min			
35 Cycles	67°	1 min 20			
Final Extension	72°	5 min			
Hold	4°				